Application Notes

Advice for setting up robust DELFIA® binding assays

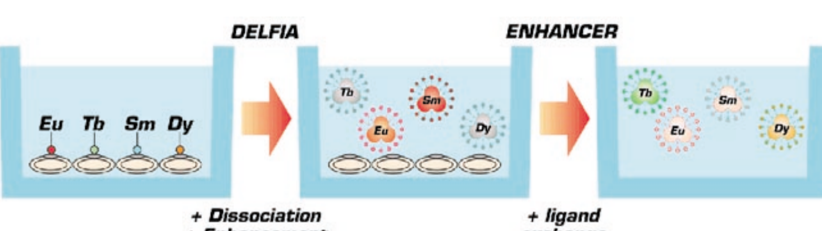
ASSAY DESIGN

DELFIA assays are separation assays built on coated microtitration plates or Acrowell filtration plates. The applications of DELFIA within drug discovery are typically in target research, assay development and lead optimization where the high sensitivity and wide dynamic range of the assays are beneficial. A unique feature of the technology is the possibility to set up multiple label assays which significantly increases the throughput of one single assay as well as reducing the reagent costs.
Typical steps of an assay are binding to a surface, washing and enhancement.
The enhancement step is a unique proprietary feature of DELFIA technology and it is the key to high sensitivity. The lanthanide labels are chelates that dissociate at low pH very rapidly in the enhancement step. The use of chelates brings some unique aspects to assay development and recognition of them is a prerequisite to development of robust DELFIA assays.

FIGURE of a typical binding assay based on the use of adherent cells growing in a microtitration well.

CONVERSION OF ELISA TO DELFIA

When converting ELISA assays to the DELFIA format use of DELFIA Assay buffer is recommended during incubation with the lanthanide tracer. Serial dilution of the key components is recommended as part of the assay optimization because the working concentration may be different using the new assay technology.
Due to the sensitivity of DELFIA technology the washing procedure may need to be optimized: Buffer should be used instead of water and a plate washer instead of manual washing. The number of wash cycles after incubation with Eu labelled reagent may need to be increased.



A coating suitable for ELISA may need to be further optimized for a DELFIA assay to achieve the benefits of the technology. Adequate binding capacity of the plate is necessary to achieve the wider dynamic range, blocking reagent should not be contaminated with heavy metals to achieve low background and the reproducibility of the coating needs to be good to get tight CV's in an assay.

DELFIA MULTILABEL ASSAYS

Key elements for success are the right choice of labels and use of high quality reagents. The Eu- and Tb-chelates are the most sensitive labels especially when using the new Yellow plate. The fluorescence signal of Sm is about 1% of the Eu-signal in DELFIA Enhancement solution. However, the difference in analyte sensitivity is not as big due to the lower background fluorescence of Sm. The lower fluorescence can thus be compensated for by using a higher amount of label per tracer or increasing the concentration of the tracer in the assay. Dual label assays are typically based on the use of Eu and Sm because they can be detected using the one Enhancement solution only. Detection of Tb and Dy requires the addition of the different type of Enhancer.
When preparing tracers for multiple label assay care must be taken to avoid contamination of the tracers with different lanthanides.

SOLID PHASE

Critical factors are the coatability of the well surface and the background fluorescence of the plate. In the first Wallac time-resolved plate fluorometers 1232 and 1234 only clear 96 well plates can be measured. In order to use other than 1244-550 microtitration plates in the 1232 and 1234 fluorometers the plate sensor may need to be inactivated. The Wallac VICTOR, is an epifluorometer which allows the use of opaque plates and 384 well plates in DELFIA assays as well.
Wallac offers microtitration plates that have been designed and tested for use in DELFIA assays. Acrowell filtration plates for ligand receptor binding assays have been added to the product range. These plates have a uniquely low Eu-fluorescence background. Plates can be coated following the general coating procedures. However, attention must be paid to produce high quality coating to be able to achieve the tight CV's characteristic of well designed DELFIA assays. Antigen coating can be very demanding and should be avoided by using streptavidin coated plates and biotinylated antigen whenever possible.
An additional critical factor is the heavy metal concentration of the reagent, e.g. bovine serum albumin (BSA) or gelatine used for saturation of the plates. Both high quality coated plates and purified stabilizer, BSA, are available from Wallac. The Stabilizer can be used also in the storage buffer of lanthanide labelled reagents.

CR84-100	Stabilizer, 7.5% BSA solution	50 ml
1244-550	DELFIA microtitration plates	8 x 12 strips, 60 plts
C554-160	Low fluorescence background 96-well plate	Solid, 60 plts
AAAND-0001	Yellow 96-well plate optimized for Sm &Tb assays	Solid, 60 plts
AAAND-0002	White 384-well plate	Solid, 50 plts
5020 *	Acrowell plate, low fluorescence background	Filtration plate, 10 plts

* Only available in USA

EU-LABELED REAGENTS

As part of the assay development serial dilution of all reagents and specially of the Eu-labeled reagent is recommended. As a rule of thumb use concentrations well below and above the concentrations used in the previous label technology.

For simple and fast DELFIA assays the use of N1-lanthanide chelates is recommended. If the Eu-labelled reagent is exposed to lower pH, higher chelate concentration or high temperature, a more stabile chelate needs to be used. In order to analyze EDTA-plasma samples in a competitive immunoassay DTPA-chelates need to be used. The chelate W2014 is specially designed for the labelling of oligonucleotides to be used in hybridization assays. During assay development LANCE Eu-W1024 labelled compounds can be used in a DELFIA assay as well as using a longer enhancement time.

Chelate type	[Chelators]	pH	Temperature	Other
DELFIA N1	< 0.05 mmol/l	> 7	< 38°C	
DELFIA W2014	< 0.2 mmol/l	> 7.5	< 100°C	[SDS] < 5%
DELFIA DTPA	< 10 mmol/l	> 6.5	< 100°C	
LANCE W1024	< 10 mmol/l	> 7	< 38°C	

ASSAY BUFFER COMPOSITION

To develop a robust DELFIA assay use of Tris-HCl based buffers at neutral or alkaline pH is recommended depending on the type of chelate. In short assay buffers containing chelating agents, e.g. HEPES and phosphate buffers can be used with N1-chelates but storage of labeled compounds in these buffers should be avoided. In cell based assays the background fluorescence can be decreased by increasing the DTPA concentration to 50 µmol/l or using 100 µmol/l EDTA in the assay buffer.

DELFIA ready for use assay buffers 1244-106, 1244-111 and 4002-0010 have been optimised for DELFIA binding assays to give minimum non-specific background. Product CR86-100 is a basic buffer concentrate that can be modified by the customer by adding assay specific blocking proteins and detergents. Product CR85-100 is a five times concentrated assay buffer without added detergents to be used in assays where detergents may interfere.

Product	Contents	Volume
1244-106 DELFIA Assay Buffer. Ready to use.	Contains BSA, bovine gamma globulins and Tween 40.	50 ml
1244-111 DELFIA Assay Buffer. Ready to use.		250 ml
4002-0010 DELFIA Assay Buffer. Ready to use.		1000 ml
CR86-100 Research Buffer Set, 2 x concentrate	BSA and detergent in separate vials.	250 ml
CR85-100 DELFIA Assay Buffer w/o detergents, 5 x concentrate	Contains BSA, bovine gamma globulins.	250 ml

ADEQUATE WASHING

Detection of lanthanides is very sensitive which easily leads to high background signal and significant sample to sample variation if washing of the plate is not adequate. Use of an automatic plate wash is necessary to obtain good reproducible results. Before addition of Enhancement solution, 4-6 washing cycles are recommended. Attention must be paid to the maintenance of the washing device in order to produce good replicates of the samples.
Since the DELFIA chelates are pH sensitive, buffered solutions like Tris-HCl with detergents are recommended for washing.

Product	Description	Volume
1244-114 DELFIA Wash concentrate	25 fold concentration of Tris- HCl buffered (pH 7.8) salt solution with Tween 20. It contains Germall II as preservative	250 ml

ENHANCEMENT STEP

For optimal fluorescence signal it is recommended that 100-200 µl of Enhancement solution be added to 96 well plates and 50 µl to 384 well plates. The recommended ways to dispense Enhancement solution are the use of a dedicated Eppendorf Multipette or a DELFIA Plate dispenser which has been optimized for slow dispensing of a solution containing detergents to avoid air bubbles. First flush the tip with Enhancement solution. Avoid dispensing Enhancement solution from glassware or from any vessel where there may have been lanthanide labelled compounds before as the sensitive solution efficiently detects any remaining lanthanides in these vessels.

	1 nM	λ em	1 nM	λ em	1 nM
Eu	–	613	1000000	613	ND
Tb	–		–	545	500000
Sm	–	643	10000	643	ND
Dy	–		–	572	10000

Table. Typical signal levels using DELFIA Enhancement solution (200 µl) and Enhancer (total volume 250 µl) for detection of lanthanides in a VICTOR time-resolved fluorometer. ND=not determined (1 second measurement time).

The plate should be measured open without tape. If the plate cannot be measured after finishing the assay it is recommended that the plate be stored empty and Enhancement solution be added prior to measurement. The plate should never be sealed with tape after addition of Enhancement solution.

The dissociation times for the different chelates vary depending upon their kinetic stability. The times given are for the DELFIA Plate shake and every shaker should be checked separately by following how long an incubation time is needed to reach a stable signal.
If vigorous shaking is used air bubbles that may interfere with the measurement can form.

Chelate type	Dissociation time
DELFIA N1	5 min
DELFIA W2014	25 min
DELFIA DTPA	30 min
LANCE W1024	15 min

The fluorescence is stable for several hours. When protected from direct sunlight and from evaporation, the signal can be read as long as 20 hours after completion of the assay. However, we recommend measurement within 1 hour as external factors may cause a decrease in signal with time, although this is extremely rare.
Tb-and Dy-labelled compounds need first to be diluted in Enhancement solution and then Enhancer should be added in the ratio of 1:4 (e.g. 50 µl Enhancer per 200 µl Enhancement solution).

1244-104 DELFIA Enhancement solution	50 ml	For detection of Eu and Sm
1244-105 DELFIA Enhancement solution	250 ml	For detection of Eu and Sm
4001-0010 DELFIA Enhancement solution	1000 ml	For detection of Eu and Sm
C500-100 DELFIA Enhancer	50 ml	For detection of Tb and Dy with DELFIA Enhancement solution

DELFIA BINDING ASSAY

This protocol is given as a possible starting point for the development of a protein binding assay.

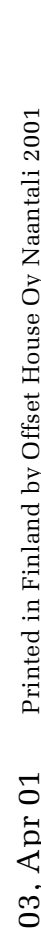
Step		Details
Wash.		Prewash the streptavidin coated plates (C122-105)
Add buffer containing biotinylated protein.		100 µl of Assay Buffer (1244-106) containing eg. 100 ng/ml of biotinylated protein
Incubate.		Slow shaking 1-2 hours RT
Wash.		Wash gently once
Add standards and samples		10 µl
Dilute tracer.		25-500 ng/ml Eu-labelled protein in Assay Buffer
Add tracer.		100 µl
Incubate		Slow shaking 60 min. RT
Wash.		4-6 washes (1244-114)
Enhance.		200 µl, slow shaking 5 min
Count.		Create counting protocol and count with VICTOR^2

MORE INFORMATION FOR OPTIMIZATION OF DELFIA BINDING ASSAYS

For analyte specific references please visit our website.
At the address lifesciences.perkinelmer.com/referencedb/
you will find more than 1000 references for the time-resolved fluorescence technologies.
Application Note 1234-965 Eu-labeled oligonucleotides are stable and sensitive as probes and primers.
Application Note 1234-966 DELFIA assays bring convenience in monoclonal antibody development: Immunoassay designs and a model assay protocol.
Application Note 1234-968 DELFIA Protein kinase assays
Application Note 1234-969 DELFIA Cell adhesion assays
Application Note 1234-976 How to optimize rapid and simple immunoassays (DELFIA)
Application Note 1420-1000 The Acrowell Plate: Low fluorescence background using the DELFIA system

Problem
Decrease in assay counts after storage of labelled compound. No counts or very low counts.
High background of the assay
Poor reproducibility of samples: How to improve precision?
Inadequate sensitivity of the assay.
Dual label immunoassay not optimized.

Possible cause/effect	Solution
* Labelled antibody stored at wrong temperature * Heavy metal contamination of BSA * Low pH of storage buffer, pH <7 * Storage buffer contains chelating agents * EDTA sample used in a one-step assay * Intrinsic characteristic of the reagents, like unstabile antibody	* Optimize storage conditions * Add purified stabilizing agents like glycerol, glucose, BSA (Wallac product CR84-100) * Eu-labelled antibody should not be exposed to chelating agents like EDTA during incubations, use two-step assays * Optimize reagents
* Background of the used plates is higher than 2000 * If after coating BG is more than two fold either solutions or instrumentation is Eu contaminated	* Change plates to 1244-550 Wallac plates, typically BG< 600 counts
* Inadequate purification of labelled antibody	* Check solutions, pipettes, wash tables etc. remove aggregates of antibodies, e.g. using Sephadex G-25 or -50 (1.5 x 30 cm) or Sepharose G-50 (1.5 x 40 cm) can be combined with Sephadex (10 cm), Superdex columns
* Presence of aggregated immunoglobulins	* After storage filter through a 0.2 µm membrane
* Inadequate washing prior to measurement	* After incubation with Eu-labelled compound 4-6 washing cycles path DELFIA Plate Wash is adequate
* Inadequate blocking of the plates	* Saturate overnight RT, or >2hours +37°C
* Inadequate washing prior to measurement * Too short an incubation with Enhancment solution	* See above * Use at least 5-10 minutes incubation on a shaker before measurement. Check for every shaker time needed for maximal signal
* Poor pipetting technique	* Dedicated Eppendorf Multipette or Dispenser is recommended for dispension of Enhance-ment Solution
* Low affinity of antibody * Uneven coating	* Immunometric assays: Increase amount of tracer per well, increase incubation time, check coating procedure etc. * Test with Wallac coated plates
* Plate sealed with tape during measurements	* Remove tape
* Trace amounts of Eu in sample, typically cell assays	* Add 50 µmol/l DTPA to assay buffer
* High background * Low maximal signal	* See above * Increase sample volume * Use two different tracer antibodies * In a sandwich assay increase antibody concentration * Big improvement possible only by changing antibody
* Low Sm-counts compared to Eu-counts * Low sensitivity of the analyte detected using Sm * Use higher Eu/Ab ratio of Sm than Eu	* Balance difference when coating e.g. 10% Ab1, 90% Ab2 * Label with europium antibody when detecting analyte requiring higher sensitivity



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